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Assessment of Lipid Peroxidation and Serum Electrolyte Profile in Epileptic Patients Exposed On Anti-Epileptic Drugs

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ABSTRACT

Background: Epilepsy is one of the most common severe disorders of the brain and 50 million people are affected by epilepsy from all over the world. In the central nervous system (CNS) electrolyte homeostasis is crucial for the brain function. Anti-Epileptic Drugs (AEDs) are neither protective nor healing but employed exclusively to control symptoms such as suppression of seizures. **Objective:** To assess lipid peroxidation and serum electrolyte profile in epileptic patients exposed on anti-epileptic drugs. **Methodology:** Fifty patients of epilepsy and ten age and sex-matched clinically apparently healthy individuals were eligible for inclusion in the study at Jinnah Hospital Lahore. 5.0 ml blood sample was taken from each individual and subjected to centrifuge at 3000-4000 rpm for 10-15 minutes for the separation of serum. The estimation of anti-oxidative biomarkers (SOD, MDA, GSH and CAT) and serum electrolyte profile ($NA^{+,}$ $K^{+,}$ Ca^{++} and Mg^{++}) were estimated. **Results:** The level of MDA in epileptic patients was increased remarkably (7.08±0.76) as compared to healthy individuals (1.67±0.19) and statistically significant (0.000<0.05). Mg^{++} level was decreased in epileptic patients (1.44±0.21) as compared to control (1.68±0.20) and statistically significant (0.003<0.05). Serum sodium (Na^{+}) level in epileptic patients was increased (1.64±9.9) as compared to control persons (1.38±5.97) and statistically significant (0.004<0.05). **Conclusion:** A relationship is present between oxidative stress, electrolyte profile and epilepsy. Lipid peroxidation level and sodium and potassium level is significantly elevated in epileptic patients as compared to control persons.

Keywords: MDA, SOD, CAT, GSH, Epilepsy, Anti-Epileptic Drugs (AEDs)

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INTRODUCTION

Epilepsy is one of the common disorders of the brain affecting 50 million people worldwide. The term epilepsy is originated from the Greek word epilambanein means to attack or seize. “Epilepsy is the name for irregular, hasty, extreme and local releases of grey matter.” The term epilepsy applied for repetitive, unfounded seizures.[1] Seizures are perceived as electromagnetic discharges in the brain in individuals liable to reputed genetic factors, intrinsic neurological disorders, and mainly unknown neurochemical mechanisms.[2] The persons that suffer from two or more arbitrary seizures, they can be known as epileptic patients.[3] Epilepsy is initiated by an increase in neuronal excitability due to changes in ion channel function, inhibitory and excitatory synaptic communication, neuronal circuitry and expression of genes encoding for critical proteins like receptors e.g. trophic factors. These variations usually occur in response to brain damage, brain malformation and genetic mutation or due to unknown causes. In developed countries, the rate of existence of epilepsy is 5.8 per 1000 and for developing countries is 10.3 per 1000.[4]

Epilepsy can be classified in three classes known as idiopathic, provoked or symptomatic. Main causes of symptomatic epilepsies are trauma, infection, deformity or a systemic genetic disease. Provoked seizures are mainly caused by specific environmental or systemic factors. Idiopathic epilepsy is primarily due to genetic reasons.[5]Oxidative stress (OS) is a condition in which the steady-state equilibrium of reactive oxygen species to anti-oxidants moves in the direction of the former triggering the potential for organic damage.[6] Predominantly, oxidative stress is defined as instability between reactive oxygen species (ROS) and reactive nitrogen species (RNS).

Electrolyte balance is essential for brain functioning in the central nervous system (CNS). Regulation of ionic stability is a process involving complex arrangement of molecules for the movement of ions. Variations in ionic gradient across cell membrane have direct or indirect effects on neuronal release and enhance epileptiform actions.[7] As potassium (K^+) currents play an important role in examining neuronal excitability, it was presumed that elevated K^+ level was the main cause of stimulating epilepsy. In some other study Role of potassium (K^+) in epilepsy is reduced significantly because potassium level was lowered as compared to occurrence of seizure.[8]

Anti-epileptic drugs (AEDs) are used to limit the symptoms such as suppression of seizures. They balance the equilibrium between neuronal excitation and inhibition. Frequent seizures are the measure of repeated and extreme hyper excitability of the central nervous system (CNS). Anti-epileptic drugs control seizures by modulating voltage-gated sodium and calcium channels, gamma-amino butyric acid (GABA) mediated inhibitory neurotransmission and reduction of glutamate-mediated excitatory neurotransmission.[9]

METHODOLOGY

Source of data

I. 50 epileptic patients were eligible for inclusion in the study at Jinnah hospital Lahore. Detailed history, clinical complications if any, habits in particular smoking and tobacco chewing were collected from subjects of the study, by giving them a questionnaire. Clinical diagnosis of the patient was also being taken into consideration.

II. 10 age and sex-matched clinically apparently healthy individuals were included as controls.

Place of work

All experimental work was done in School of Medical Lab Technology department, Minhaj University Lahore and was approved by the Research and Ethical committee of Minhaj University Lahore.

Method of collection of data

Blood samples were collected with aseptic precaution. Informed consent from subjects was obtained before collection of blood samples.

Sample and sampling technique

Blood samples of Patients and controls were collected and processed. 5ml blood was collected in EDTA containing Vaccutainers and centrifuged and aliquoted the serum.

Chemicals: All chemical reagents of analytical grades were purchased from Sigma Chemical Co. (St. Louis, Mo, USA).

Following parameters were estimated

Biochemical assays for oxidative stress profile

Catalase (CAT) was assayed according to the method of Aebi, 1974[10]. Malondialdehyde (MDA) biomarker of Lipid peroxidation in liver tissues was estimated calorimetrically by measuring Thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa *et al.*, 1979[11]. Superoxide dismutase (SOD) activity was determined by the method of Kakkar *et al*., 1972[12] and Glutathione (GSH) was determined by the method of Moron *et al*., 1979[13].

Estimation of serum electrolyte profile

Serum Electrolyte profile (Na^+, K^+, Ca^{++} and Mg^{++}) was determined by the use of flame photometer.

RESULTS

The results presented in table 1 (given below) show the picture of the different parameters estimated in the epileptic patients receiving Anti-Epileptic Drugs (AEDs). When the MDA (biomarker of lipid peroxidation) level was measured in epileptic patients, it was observed that it was elevated remarkably in the patients (7.08±0.76) as compared to healthy individuals (1.67±0.19). SOD level in the epileptic patients was (0.17±0.11) while in control it was (0.59±0.13) so it was decreased in the patients as compared to control objects. When the Glutathione (GSH) level was measured it was observed that GSH level was decreased remarkably (2.21±0.78) as compared to healthy objects (8.54±1.44). Serum level of Catalase (CAT) in the epileptic patients was also decreased as it was (0.80±0.58) while in the control was (3.75±0.86). Similarly when the serum electrolyte profile (Na^+, K^+, Mg^{++} and Ca^{++}) was measured in epileptic patients, it was noticed that serum Potassium (K^+) level was increased (6.18±1.05) as compared to healthy ones (4.09±0.52). Calcium (Ca^{++}) level was decreased in epileptic patients (7.52±1.17) as compared to control subjects (8.71±0.85). Similarly when the Na^+ level was measured it was noticed that serum Na^+ level was elevated in patients (1.64±9.9) as compared to healthy individuals (1.38±5.97). The Mg^{++} level in the epileptic patients was decreased

(1.44±0.21) as compared to the healthy objects (1.68±0.20). It was also noticed that all the parameters of serum electrolytes were statistically significant (P<0.05).

Table 1: Comparison of different parameters in epileptic patients receiving anti-epileptic drugs (AEDS) with control.

Parameters	Control (n=10)	Patients (N=50)	P < 0.05
MDA	1.67±0.19	7.08±0.76	0.000
SOD	0.59±0.13	0.17±0.11	0.000
GSH	8.54±1.44	2.21±0.78	0.000
CAT	3.75±0.86	0.80±0.58	0.000
Potassium (K^+)	4.09±0.52	6.18±1.05	0.000
Calcium (C^{++})	8.71±0.85	7.52±1.17	0.070
Sodium (Na^+)	1.38±5.97	1.64±9.9	0.004
Magnesium (Mg^{++})	1.68±0.20	1.44±0.21	0.003

The results presented in table 2 (given below) show the role of different parameters in the epileptic patients according to the gender. MDA level in male epileptic patients was (6.05±2.33) while in females (5.94±2.29). When the SOD level was measured it was observed that SOD level in males was (0.24±0.19) and in females it was (0.28±0.21).

Table 2: Comparison of different parameters in epileptic patients (according to gender) receiving anti- epileptic drugs (AEDS).

Parameters	Females (n=24)	Males (n=26)	P <0.005
MDA	5.94±2.29	6.05±2.33	0.874
SOD	0.28±0.21	0.24±0.19	0.516
GSH	3.55±2.88	3.42±2.60	0.865
CAT	1.35±1.29	1.43±1.43	0.840
Potassium (K^+)	5.92±1.25	5.62±1.32	0.417
Calcium (Ca^{++})	7.87±1.17	7.65±1.24	0.523
Sodium (Na^+)	1.59±13.65	1.59±15.00	0.991
Magnesium (Mg^{++})	1.50±0.22	1.47±0.24	0.664

Serum GSH level in male epileptic patients was (3.42±2.60) and in female epileptic patients was (3.55±2.88). The level of Catalase (CAT) in males was (1.43±1.43) as compared to females (1.35±1.29). The serum Potassium (K^+) level in females was (5.92±1.25) and in males was (5.62±1.32). Similarly Calcium (Ca^{++}) level in male epileptic patients was (7.65±1.24) and in females (7.87±1.17). When the Na^+ level was measured in female epileptic patients it was (1.59±13.65) while in males (1.59±15.00). Mg^{++} level in males was (1.47±0.24) while in females it was (1.50±0.22). It was also observed that all the parameters were statistically non-significant (P>0.05) which showed that in the progression of the epilepsy, gender does not matter. It can be present in both either males or females.

DISCUSSION

In current study, data was taken of 50 epileptic patients out of whom 24 were females and 26 males of different ages receiving anti-epileptic drugs. Oxidative stress profile including MDA, CAT, SOD and GSH was estimated and electrolyte profile including (Na^+, K^+, Ca^{++} and Mg^{++}) was also measured and comparison with gender was done of patients with controls. Unsaturated fatty acids combine to form lipid membranes which particularly are sensitive to oxidative stress and peroxidation of the lipids in the membranes, and damage the cell membranes. Oxidative stress has more damaging effect on nervous tissues, due to the high concentration of polyunsaturated fatty acids that are susceptible to lipid peroxidation and generate free radicals. Lipid peroxidation increases the level of MDA, it would have implicated in the development of epilepsy. It receives a large percentage of oxygen and is relatively deficient in antioxidant enzymes such as Catalase (CAT) Glutathione (GSH) and Super oxide dismutase (SOD) which are may be a cause of epilepsy. In presents study Malondialdehyde (MDA) was raised in epilepticus when compared with controls. Elevated level of MDA causes oxidative stress in patients, damages the lipid membrane and leading epilepsy. Current results also show that oxidative stress profile including MDA, CAT, SOD and GSH has no more significant difference in male and female data suggesting statistically non-significance. Present data shows that the level of the lipid peroxidation is significantly higher in epileptic patients as compared to the healthy ones but the oxidative stress has no significant difference in the males and females.

Serum Electrolyte profile of 50 epileptic patients receiving anti-epileptic drugs was measured by the

use of flame photometer. It was found much variations in Cat^{+} ions concentrations in patients as compared to controls. Electrolyte homeostasis in the central nervous system (CNS) is essential for brain function. Serum Electrolyte abnormalities may affect the sensorium. Acute or severe electrolyte imbalances frequently cause epileptic seizures. Epileptic seizures are especially common in patients with sodium disorders, potassium disorders, hypocalcemia, and hypomagnesemia.

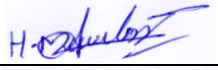
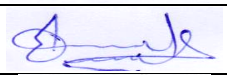
CONCLUSION

It is concluded in current study that there is a strong relationship between oxidative stress, serum electrolytes disturbance and epilepsy. Biochemical study of the epilepsy suggested that oxidative stress and electrolyte profile play a key role in the progression of epilepsy. It may also concluded that the level of the lipid peroxidation is significantly elevated in epilepsy as compared to the control as a result MDA level was remarkably increased and antioxidant enzymes decrease. Electrolyte profile also varies in epilepticus. It is evaluated in present study that the values of Na^{+} and K^{+} were increased and Ca^{++} and Mg^{++} were decreased significantly in epileptic patients.

REFERENCES

1. Shorvon SD. The etiologic classification of epilepsy. Epilepsia. 2011;52(6):1052-7.
2. Ngugi AK, Bottomley C, Kleinschmidt I, Sander JW and Newton CR. Estimation of the burden of active and lifetime epilepsy: a meta-analytic approach. Epilepsia. 2010;51(5):883-90.
3. Kobau R and Price P. Knowledge of epilepsy and familiarity with this disorder in the U.S. population: Results from the 2002 Health Styles survey. Epilepsia. 2003;44(11):1449-54.
4. Bazil CW, Morrell MJ and Pedley TA. Epilepsy. Neurology. 2005;11:990-1008.
5. Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology. Epilepsia. 2010;51:676-85.
6. Stamler JS, Simon DJ and Jaraki O. S-nitrosylation of tissue-type plasminogen activator confers vasodilatory and antiplatelet properties on the enzyme. Proc Natl Acad Sci U S A. 1992;89(17):8087-91.
7. Scwartzkroin PA, Baraban SC and Hochman DW. Osmolarity, ion flux, and changes in brain excitability. Epilepsy Res. 1998;32(1-2):275-85.
8. Somjen GG. Extracellular potassium in the mammalian central nervous system. Annu Rev Physiol. 1979;41:159-77.
9. Marson A, Jacoby A, Johnson A, Kim I, Gamble C and Chadwick D. Immediate versus deferred antiepileptic drug treatment for early epilepsy and single seizures: a randomized controlled trial. Lancet. 2005;365(9476):2007-13.
10. Aebi H. Catalase in Bergmeyer HU (eds). Methods in Enzymatic Analysis. New York, Academic Press. 1974;3:276-86.
11. Ohkawa H, Ohishi N and Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. J Anal Biochem. 1979;95:351-8.
12. Kakker P, Das B and Viswanathan PN. A modified spectrophotometer assay of superoxide dismutase. Ind J Biochem Bio. 1948;21:130-32.
13. Moron MS, Depierre JW and Mannervik B. Levels of glutathione reductase and glutathione S transferase in rat lung and liver. Biochem Biophysic Acta. 1979;582:67-8.

AUTHORSHIP AND CONTRIBUTION DECLARATION

Name of Author	Contribution to the paper	Author's Signatures
Dr. Hafiz Muhammad Arsalan	To collect data and design the project, perform practical	
Dr. Zeemal Seemab Amin	To perform practical	
Dr. Abdur Rauf Hammad	Design and analyze the project